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Review Article

Endothelium-derived relaxing and contracting factors: potential role in coronary artery disease

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Endothelial cells can release substances which profoundly affect vascular tone and platelet function. The inhibitory substances include endothelium-derived relaxing factor (EDRF or nitric oxide), prostacyclin and probably an endothelium-derived hyperpolarizing factor. Endothelin is a potent vasoconstrictor peptide released from endothelial cells. Under certain conditions, the endothelium can also produce angiotensin II, thromboxane A_2 and a cyclooxygenase-dependent endothelium-derived contracting factor. In normal arteries, the effects of EDRF appear to dominate. In diseased arteries, the release and action of EDRF is impaired and that of endothelium-derived contracting factors is increased. Hyperlipidaemia, atherosclerosis and hypertension reduce endothelium-dependent relaxations. Hypoxia inhibits the release of EDRF and prolonged ischaemia severely impairs the response. Regenerated endothelium at sites of mechanical injury exhibits selective defects in response to aggregating platelets. The more effective release of EDRF in arterial compared with venous bypass grafts further suggests an involvement of the factor in preventing vascular occlusion. Therapeutic interventions with specific drugs and diets can augment the impaired endothelium-dependent relaxation of diseased arteries. Thus, functional changes of the endothelium in coronary artery disease may be an important factor in the development of vasospasm, ischaemia and thrombosis.

Introduction

Reduced blood flow to the myocardium is the common feature of ischaemic heart disease[1]. Ischaemia may develop due to vascular thrombosis, stenosis and/or vasospasm[2–4]. In most instances, the major event in myocardial infarction is occlusion of one or more large epicardial coronary arteries. Plaque fissuring, coronary vasospasm and thrombus formation are crucial events in unstable angina and evolving myocardial infarction. Similarly, increased platelet vessel wall interactions with platelet deposition play an important role in coronary bypass graft occlusion[5]. In exercise-induced angina, vascular tone of the diseased arterial segment can modulate the degree of myocardial ischaemia[6]. Recent research indicates that the endothelium has a protective function in the circulation by preventing vasospasm and thrombus formation. This review summarizes current knowledge on the physiological importance of endothelium-derived relaxing and contracting factors and their potential role in coronary artery disease[7].

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Endothelium-derived vasoactive substances

ENDOTHELIUM-DERIVED RELAXING FACTORS

Prostacyclin (PGI_2) is the major cyclooxygenase product of the blood vessel wall[8]. Endothelial cells produce 10–20 times more PGI_2 than do vascular smooth muscle cells (Fig. 1). The concentration of PGI_2 synthetase progressively decreases from the intimal to the adventitial side of the blood vessel wall. Thus, the endothelium is a major source of the

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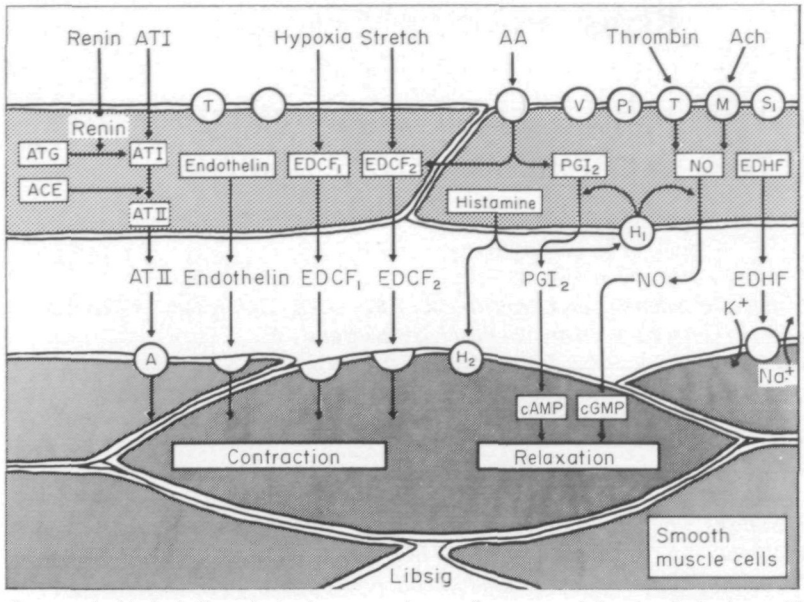

Figure 1 Endothelium-derived vasoactive substances. AA = Arachidonic acid; ACE = angiotensin converting enzyme; ATG = angiotensinogen; ACh = acetylcholine; ATI/II = angiotensin I/II; cAMP/cGMP = cyclic adenosine/guanosine monophosphate; EDCF = endothelium-derived constricting factor(s); EDHF = endothelium-derived hyperpolarizing factor; NO = nitric oxide; PGI_2 = prostacyclin (modified from ref. 7).

luminal release of the prostanoid, but its production is not restricted to these cells. PGI_2 is a potent inhibitor of platelet aggregation and a vasodilator of coronary arteries. Its mechanism of action involves increases in intracellular cyclic adenosine 3′,5′-monophosphate (cyclic AMP). Platelet-inhibitor drugs, such as aspirin, which inhibit cyclooxygenase, reduce vascular prostacyclin production.

Furchgott and Zawadzki[9] first demonstrated that the relaxation in arteries induced by acetylcholine is endothelium-dependent and mediated by a diffusible substance with a half-life of a few seconds which they named *endothelium-derived relaxing factor (EDRF*, Fig. 2). Haemoglobin, oxygen-derived free radicals and antioxidants are potent inhibitors of EDRF[10–12]. The factor is a potent vasodilator and inhibitor of platelet adhesion and aggregation (Fig. 3)[13–15]. Subthreshold concentrations of either EDRF or PGI_2 enhance the vascular and antiaggregatory effects of the other substance[13,16]. In the coronary circulation, EDRF preferentially increases subendocardial blood flow[17]. The action of EDRF on vascular smooth muscle involves activation of soluble guanylate cyclase and in turn increases in intracellular levels of cyclic guanosine 3′,5′-monophosphate (cyclic GMP) (Fig. 1)[18]. Substances interfering with the production of cyclic GMP (methylene blue, LY 83583) inhibit endothelium-dependent relaxations. Endothelial cells in culture release nitric oxide after stimulation with bradykinin[19]. Nitric oxide has a similar half-life and mode of action to EDRF and the amounts released from endothelial cells appear sufficient to explain the biological activity of the factor in vascular tissues and in platelets[19]. L-arginine is the precursor substance from which nitric oxide is cleaved in endothelial cells[20]. Thus, the endothelium produces an endogenous nitrate which causes relaxation of vascular smooth muscle and inhibition of platelet function. EDRF is released in response to flow (shear stress), platelet-derived products (i.e. adenosine diphosphate, thrombin, serotonin) and hormones and autocoids (i.e. bradykinin, histamine, noradrenalin, substance P and vasopressin)[7,12,21,22–24]. Aggregating platelets release enough adenine nucleotides and serotonin to induce endothelium-dependent relaxations in isolated blood vessels[23]. Adenosine diphosphate is the main mediator of the response to aggregating platelets in human coronary arteries, while the effects of serotonin and thromboxane A_2 are endothelium-independent (Fig. 3)[24]. Several platelet-derived

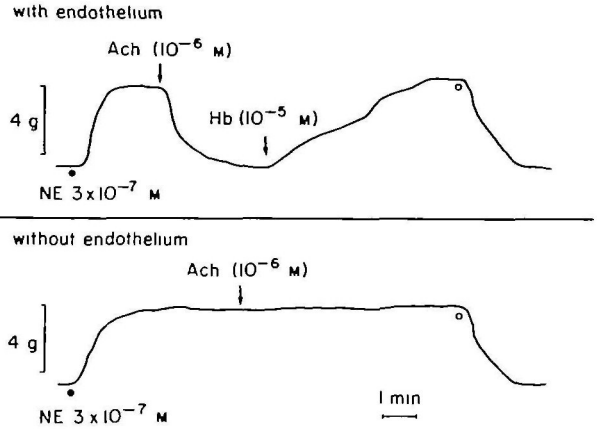

Figure 2 Endothelium-dependent relaxation in response to acetylcholine in a human internal mammary artery (NE; original recording). The relaxation in response to acetylcholine can be reversed by haemoglobin (Hb 10^{-5} M; upper panel) (from ref. 10, by permisson).

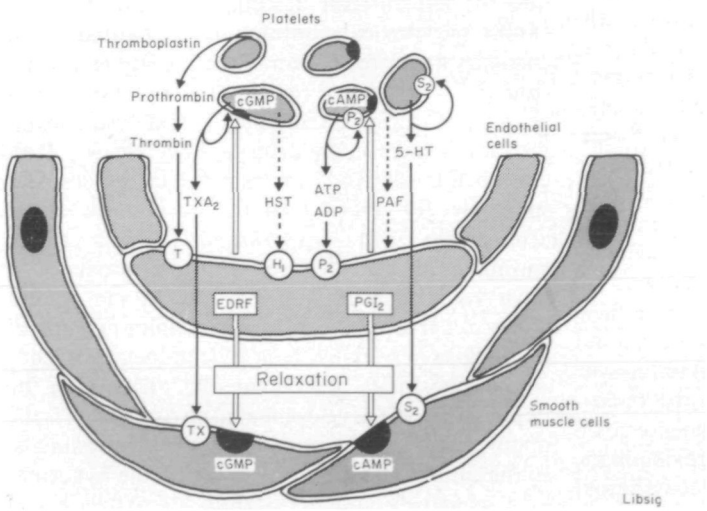

Figure 3 Release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2) by platelet-derived substances such as thrombin, adenosine tri- and diphosphate (ATP/ADP) and by histamine (HST) in human arteries. EDRF and PGI_2 cause vascular relaxation and inhibit platelet function. - - - = uncertain (modified from ref. 7).

products stimulate the endothelial production of EDRF as well as that of PGI_2 and tissue plasminogen activator[7]. Thus, at sites where platelets are activated, potent vasodilators and inhibitors of platelet function are released which may provide a protective mechanism against vasospasm, ischaemia and thrombus formation.

In addition, the endothelium must release an *endothelium-derived hyperpolarizing factor (EDHF*; Fig. 1)[25]. The sodium–potassium ATPase in-

hibitor, ouabain, reduces endothelium-dependent relaxations to acetylcholine in the canine femoral artery[26]. Acetylcholine causes endothelium-dependent increases in membrane potential of vascular smooth muscle cells which can be blocked by ouabain[27]. Exogenous nitric oxide does not hyperpolarize vascular smooth muscle cells nor does haemoglobin prevent the increase in membrane potential in response to acetylcholine, indicating that an endothelium-derived substance other than EDRF must be involved[28]. Although the physiological role of this factor is unclear yet, hyperpolarization of vascular smooth muscle cells may contribute to the sustained phase of endothelium-dependent relaxations and it may render the cells less responsive to contractile stimuli.

ENDOTHELIUM-DERIVED CONTRACTING FACTOR(S)

Under certain conditions, the endothelium can mediate vasoconstriction (Fig. 1)[7]. Yanasigawa *et al.*[29] have recently described the vasoconstrictor peptide *endothelin* which is released from endothelial cells in culture. Endothelin is the most potent endogenous vasoconstrictor substance known, with a half-maximal effective concentration 1–2 orders of magnitude lower than that of any other known cardiovascular hormone. Thrombin and the calcium ionophore, A23187, express preproendothelin messenger RNA in cultured endothelial cells and in large arteries in situ. The peptide binds to specific membrane receptors of vascular smooth muscle cells where it induces increases in intracellular calcium and in turn long-lasting contractions[30]. Whether the endothelium-derived contracting factor released under hypoxic conditions in the canine coronary artery is endothelin or an as yet unknown substance is uncertain[31]. In addition, the endothelium and vascular wall possess an independent renin–angiotensin system which may provide high local concentrations of the potent vasoconstrictor *angiotensin II*[32]. *Histamine, thromboxane* A_2 and another *cyclooxygenase-dependent endothelium-derived contracting factor* can also be produced in endothelial cells of certain vascular beds (Fig. 1)[7].

Thus, the endothelium profoundly affects vascular smooth muscle tone and platelet function by the release of endothelium-derived relaxing and contracting factors (Fig. 1). Under physiological conditions, endothelium-derived relaxing factors most likely dominate. Indeed, in normal human conduit arteries and in mesenteric resistance arteries of the rat, EDRF overrides endothelin[33,34]. An alteration of the release of endothelium-derived relaxing and/or contracting factors under pathological conditions, on the other hand, may be important in the pathophysiology of cardiovascular disease.

Cardiovascular risk factors and the endothelium

Risk factors for coronary artery disease such as hyperlipidaemia, atherosclerosis and hypertension impair endothelium-dependent relaxations in experimental animals and in human coronary arteries. In the rabbit aorta, *low-density lipoprotein* (LDL), but not high-density lipoprotein (HDL), inhibits endothelium-dependent relaxations in response to acetylcholine[35]. The inhibitory concentration of the lipoprotein corresponds to that found in plasma of patients with severe hyperlipidaemia. In addition, LDL and *atherosclerosis* inhibit vascular prostacyclin production[36]. In atherosclerotic arteries, including the human coronary artery, endothelium-dependent relaxations in response to acetylcholine, adenosine diphosphate, bradykinin, substance P and aggregating platelets are reduced (Fig. 4)[24,37–41]. Selective infusion of acetylcholine into the left anterior descending coronary artery causes paradoxical contractions in patients with coronary artery disease and in transplant recipients (Fig. 5)[40]. A reduced release of EDRF, structural changes of the intima and media, and — at least in the porcine coronary artery — the release of an endothelium-derived contracting factor are responsible for these changes in atherosclerotic arteries[39,41]. In *experimental hypertension*, endothelium-dependent relaxations are reduced in most vascular beds (Fig. 6)[7,42,43]. In the carotid artery of hypertensive rats, endothelium-dependent relaxations in response to acetylcholine and adenosine diphosphate are blunted[42]. This may be important in the development of stroke, which is the major cardiovascular complication of these animals. In the canine coronary circulation, acute hypertension selectively and persistently attenuates endothelium-dependent responses to serotonin.[43] Contractions in response to endothelin are enhanced in renal, but not in mesenteric resistance arteries of hypertensive rats[33,34]. Endothelin almost completely inhibits the effects of EDRF in hypertensive, but not in normotensive arteries.[33]

Ischaemia, myocardial infarction and the endothelium

In vitro, hypoxia and ischaemia inhibit the production and release of endothelium-derived relaxing factor and — at least in the canine coronary

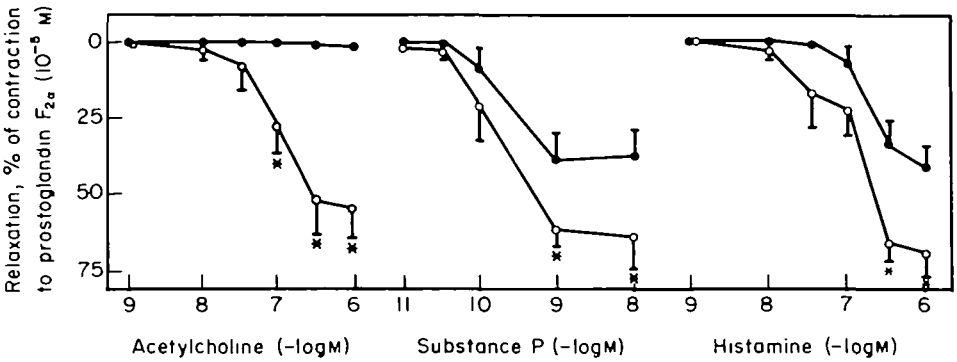

Figure 4 Endothelium-dependent relaxations in response to acetylcholine, substance P, and histamine in normal (○) and atherosclerotic (●) human coronary arteries. Note the impaired response in diseased arteries (●). *Denotes statistically significant difference ($P < 0{\cdot}05$) (modified from ref. 39, by permission).

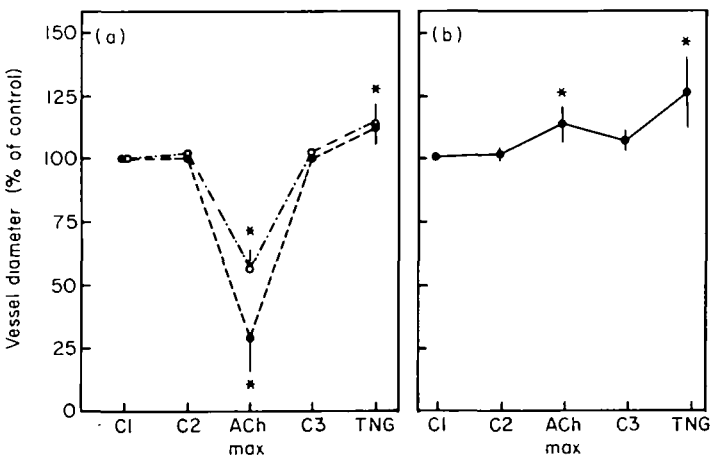

Figure 5 Effects of intracoronary infusion of acetylcholine on the diameter of the left anterior descending coronary artery in (a) patients with coronary artery disease and (b) normal subjects. Note the paradoxical contractions of the diseased arteries. Ach = acetylcholine; C1, C2, C3 = control period, vehicle control and repeated control, respectively; TNG = nitroglycerin (modified from ref. 40, by permission). ● - - - ● = prestenotic segment; ○ - - - ○ = stenotic segment; ●——● = normal vessel.

artery — trigger the release of an endothelium-derived contracting factor[31,45]. In some vascular beds, endothelial prostaglandins contribute to hypoxic vasodilatation[46]. After experimental *myocardial infarction* with delayed reperfusion, endothelium-dependent relaxations of the canine coronary artery to acetylcholine and thrombin are severely impaired and the contractions induced by the enzyme are enhanced[47,48]. Pretreatment of the animals with the calcium-channel antagonist, verapamil, partially restores endothelium-dependent relaxations, indicating that accumulation of intracellular calcium during ischaemia is involved in endothelial cell injury. In addition to the ischaemic injury, reperfusion itself may traumatize the endothelium[49]. Reperfusion injury most likely is related to the formation of free radicals and/or enhanced neutrophil-endothelial interactions[49]. Indeed, oxygen-derived free radicals destroy EDRF and they are cytotoxic[11]. Reperfusion injury and the decreased release of EDRF associated with it can at least in part be prevented by intracoronary

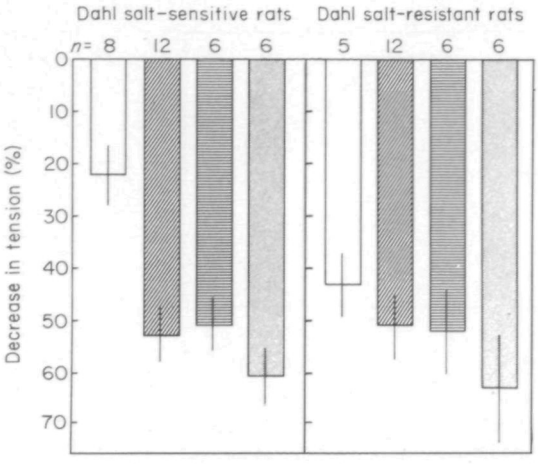

Figure 6 Effect of antihypertensive therapy on endothelium-dependent relaxations in response to thrombin IU ml^{-1} in the aorta of Dahl salt-sensitive (left) and salt-resistant (right) rats on a diet containing either 8% or 0·1% NaCl. Note the normalization of the response to thrombin in salt-sensitive rats on 8% NaCl (i.e. hypertensive group) during antihypertensive therapy (AHD) (from ref. 78, by permission of the American Heart Association). □ = 8% NaCl. ▨ = 0·1% NaCl; ▤ = 8% NaCl + AHD (2 weeks); ▥ = 8% NaCl + AHD (8 weeks).

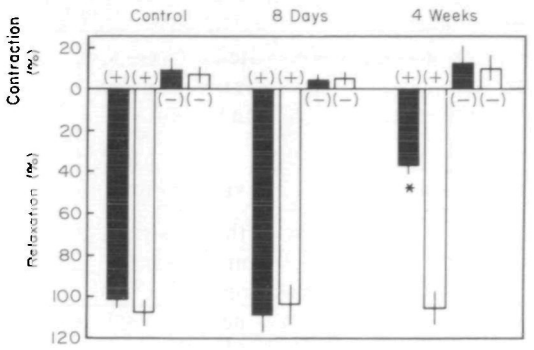

Figure 7 Endothelium-dependent relaxations to aggregating platelets in porcine coronary arteries 1 and 4 weeks after deendothelialization with a balloon catheter. Note the impaired relaxations four weeks after the intervention. ■ = LAD; □ = LCX; (+) = with endothelium; (−) = without endothelium; * = statistically significant difference from control ($P < 0.05$); n = 6 in each group (from ref. 63, by permission of the American Heart Association).

infusion of a perfluorochemical[49]. Reperfusion-induced endothelial dysfunction appears to persist as does that after mechanical injury (see section on endothelium dysfunction and vascular occlusion)[50].

Endothelium-dependent responses and coronary vasospasm

Coronary artery spasm can contribute to myocardial ischaemia[2,51,52]. A local dysfunction of the endothelium may play a role in that phenomenon. Indeed, coronary spasm can be provoked by a variety of substances which release EDRF in isolated arteries, such as acetylcholine, methacholine, histamine, serotonin and ergonovine[53,54]. All these substances exert endothelium-dependent inhibitory and direct excitatory effects. Thus, a local loss of EDRF and/or the release of an endothelium-derived contracting factor may precipitate coronary spasm in response to these substances. Intracoronary *acetylcholine* produces paradoxical contractions in patients with coronary artery disease (Fig. 5)[40]. *Histamine* causes vasodilatation when infused into the normal coronary circulation and endothelium-dependent relaxations in isolated human arteries[55–57]. Removal of the endothelium unmasks contractions in response to the amine. In the pig, coronary vasospasm can be provoked by histamine at sites of previous endothelial denudation in conjunction with an atherogenic diet[58]. A local increase in adventitial mast cells, which are a major source of histamine, has been documented in a patient with coronary spasm[59]. Coronary arteries of patients with coronary artery disease contain increased amounts of histamine and exert enhanced contractions in response to the amine[60]. Thus, at sites of previous endothelial injury and dysfunction, the direct vasoconstrictor effects of the amine may predominate and precipitate vasospasm. However, histamine is effective in provoking vasospasm only in some, but not all patients with variant angina[54].

Consistent with platelet activation, coronary sinus thromboxane B_2 levels are elevated during attacks of variant angina[61]. Thus, platelet-derived substances such as serotonin, adenosine diphosphate and thrombin are abundantly present and may contribute to the contraction of the diseased arterial segment. In vivo endothelial denudation of the coronary artery leads to spontaneous contraction and enhanced vasoconstrictor responses to *serotonin*[62,63]. Porcine coronary arteries with regenerated endothelium after balloon injury exhibit a selective and persistent defect of endothelium-dependent relaxations in response to serotonin and aggregating platelets (Fig. 7)[63]. In addition, contractions induced by serotonin are enhanced in pig coronary artery rings with regenerated endothelium, suggesting the release of an endothelium-

derived contracting factor. In man, the defect would have to involve a platelet-derived mediator other than serotonin, since the monoamine does not induce the release of EDRF in human arteries[24,57].

Endothelium dysfunction and vascular occlusion

In normal blood vessels, the release of EDRF, prostacyclin and tissue plasminogen activator from endothelial cells may help prevent vasospasm and thrombus formation. In atherosclerotic arteries, platelets become activated indicating an increased *platelet-vessel wall interaction.* Coronary sinus blood from patients with coronary artery disease exhibits vasoconstrictor activity in isolated arteries and this effect is related to the severity of the disease[64]. Endothelial dysfunction may be a crucial factor allowing platelet deposition and activation, release of platelet-derived substances and, in turn, decrease in local blood flow.

In man, increased platelet–vessel wall interactions are particularly important in the pathophysiology of *unstable angina.* Coronary angioscopy in patients with this syndrome reveals complex plaques with intimal haemorrhage and thrombi, while these findings are lacking in patients with stable angina[65]. At the site of intravascular haemorrhage, free haemoglobin may inhibit EDRF. In the dog, thromboxane A_2 receptor antagonists inhibit cyclic variations in flow as well as platelet deposition in stenosed coronary arteries[66]. This indicates that there is a continuous build-up and flush-away of platelet clots interfering with local blood flow. Endothelial injury of a large conduit artery provokes collateral arterial vasoconstriction mediated by serotonin and thromboxane A_2 released from platelets adhering and aggregating at the site of endothelial denudation[67]. Substances interfering with either the production or the vascular effects of platelet-derived products (i.e. cyclooxygenase inhibitors, calcium-channel antagonists), on the other hand, improve symptoms or the incidence of subsequent myocardial infarction in patients with unstable angina[68–70]. This indicates that altered intimal surface characteristics, most likely a decreased release of EDRF and PGI_2 at sites of atherosclerosis and intimal bleeding, are associated with platelet activation and deposition and the release of platelet-derived substances. All these events favour vasoconstriction of epicardial coronary arteries as well as of collaterals, and thrombus formation.

Arterial and venous coronary *bypass vessels* may provide a model to study possible mechanisms involved in vascular occlusion in vitro in human tissue. As a graft, the internal mammary artery has remarkedly higher patency rate than the saphenous vein. Since this difference persists, if grafts supplying the same vascular bed (i.e. left anterior descending coronary artery) are compared, this indicates that different biological properties of arterial and venous bypass vessels must be involved[71]. In mammary arteries, acetylcholine induces complete endothelium-dependent relaxations and induces a marked rise in cyclic GMP levels in vascular smooth muscle cells (Fig. 2)[10,72]. The response evoked by the muscarinic agonist is unaffected by indomethacin, but inhibited by methylene blue and haemoglobin, identifying EDRF as the mediator. In contrast to the mammary artery, saphenous veins exhibit much weaker endothelium-dependent relaxations in response to acetylcholine (Fig. 8)[10]. However, veins can respond to nitrovasodilators and exogenous nitric oxide as well as to arterial EDRF transferred from a mammary artery segment with endothelium[10]. Indeed, the sensitivity to nitric oxide is enhanced in human veins compared with arteries indicating a reduced release of EDRF in the former[72]. This may also explain why nitrates used in the treatment of angina pectoris have a more prominent effect on veins and preload than on vascular tone of coronary arteries. The release of EDRF in response to thrombin and adenosine diphosphate in the mammary artery provides arterial grafts with protective mechanisms against vasospasm and platelet adhesion and aggregation, which are weak or absent in the vein. Spasm of coronary bypass grafts has been documented angiographically[73,74]. Chronically implanted human saphenous vein grafts contract in response to a variety of vasoconstrictor hormones in vitro[75]. A decreased endothelial release of EDRF and PGI_2[76] would be associated with platelet activation and adhesion to the vessel wall, and this has been implicated in the etiology of venous graft occlusion[5]. Indeed, inhibition of platelet function does enhance the patency rate of venous grafts which lack this protective mechanism[5].

Endothelium-derived contracting factors such as endothelin dominate EDRF in venous, but not in arterial grafts[34]. In addition, histamine evokes endothelium-dependent contractions in the saphenous vein, but endothelium-dependent relaxations in the mammary artery[57].

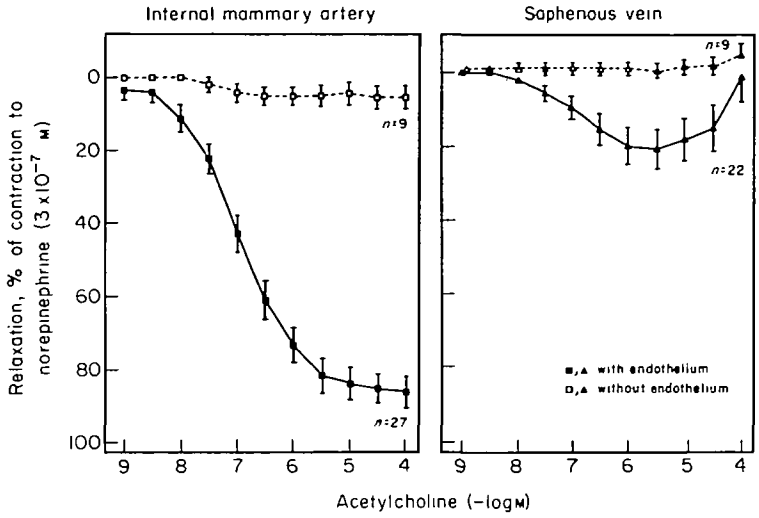

Figure 8 Endothelium-dependent relaxations in response to acetylcholine in internal mammary arteries (left), saphenous veins (right) obtained from patients undergoing coronary bypass surgery. Note the much more pronounced response in arterial than in venous grafts (from ref. 10, by permission). Closed symbols = with endothelium; open symbols = without endothelium.

Therapeutic implications

Functional changes of the endothelium in atherosclerosis and hypertension are at least partly reversible[77–80]. In the aorta of cholesterol-fed rabbits, treatment with the phosphodiesterase inhibitor, *dipyramidole*, augments or normalizes the impaired endothelium-dependent relaxations to acetylcholine and adenosine triphosphate[79]. Similarly, non-hypotensive dosages of *calcium-channel antagonists* — which suppress the development of atherosclerosis — partially restore endothelium-dependent relaxations in response to acetylcholine[80]. Eighteen months after cessation of an atherogenic diet, endothelium-dependent relaxations normalize in the iliac artery of atherosclerotic primates[77]. In the aorta of rats with salt-induced hypertension, antihypertensive therapy normalizes blood pressure and restores the blunted endothelium-dependent relaxations to acetylcholine, adenosine diphosphate and thrombin (Fig. 6)[78].

On the other hand, dietary components which are known to protect from coronary artery disease and stroke, improve endothelial function. Populations with a high dietary fish consumption exhibit a low death rate from coronary artery disease[81]. In the pig, the development of coronary atherosclerosis can be delayed by dietary cod-oil supplementation[82]. Dietary supplementation with fish-oil or its active component, eicosapentaenoic acid, markedly enhances endothelium-dependent relaxations in response to aggregating platelets and platelet-derived substances, such as adenosine diphosphate, serotonin and thrombin, in isolated porcine coronary arteries and augments the release of EDRF from cultured endothelial cells[83,84]. In addition, fish oil inhibits platelet function, reduces endothelial adherence of white blood cells and lowers plasma lipids[7,81]. High dietary potassium, which is associated with a low incidence of stroke, augments endothelium-dependent relaxations in hypertensive rats[85].

Platelet inhibitory drugs which inhibit cyclooxygenase (i.e. aspirin) not only block the production of thromboxane A_2, but also that of vascular PGI_2[8]. The inability of these drugs to inhibit EDRF may at least in part explain why even high dosages did not exert a thrombogenic effect in patients[86]. Preserved vascular PGI_2 production in patients under antiplatelet drugs may be important, since the prostanoid augments the vascular and antiaggregatory effectsof EDRF[8,13,16]. Thus, lower or alternate daily dosages (i.e. 50–100 mg) are probably more effective in preventing vascular occlusion than are the higher dosages used in most trials[86].

In disease states with a reduced production of EDRF, substitution with an *EDRF-like drug* might

be promising. Nitroglycerin differs from the endogenous nitrate EDRF in many respects, among them rapid tolerance and only weak inhibition of platelet function[87]. Whether or not chronic nitroglycerin therapy attenuates the response to EDRF is uncertain[88,89]. SIN-1, the active metabolite of the nitrate molsidomine, has remarkable similarities with EDRF. It directly activates soluble guanylate cyclase, it induces vascular relaxation with a potency similar to that of EDRF, inhibits platelet function, and tolerance does not seem to occur during chronic therapy[88,90,91]. Long-term studies are needed to assess possible vascular protective effects of this EDRF-like nitrate.

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